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Review

Metabolic Engineering of *Escherichia coli* for the Production of L-Homoserine

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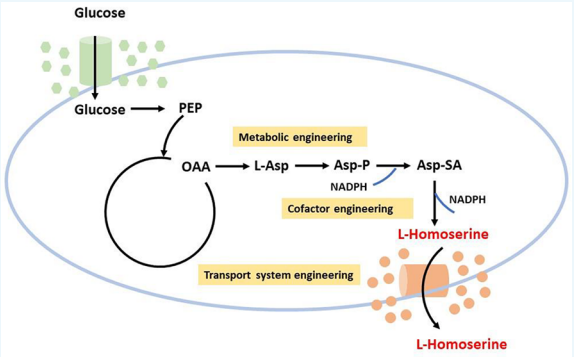
ABSTRACT: L-Homoserine embodies significant functional properties as an amino acid of utmost importance, showcasing remarkable utility within the industrial realm. As synthetic biology and biotechnology continue to advance, the synthesis of L-homoserine through microbial fermentation emerges as a compelling and eco-conscious approach. This Review summarized the recent progress in systematic metabolic engineering strategies for improving L-homoserine production in *Escherichia coli*, including blocking the competing and degrading pathways, strengthening the key enzymes and precursors, and genetic modification of transport systems. We discussed and compared these systematic metabolism strategies, which have laid a solid foundation for the microbial industrial production of L-homoserine.



KEYWORDS: *L-homoserine production, Escherichia coli, metabolic engineering, L-homoserine biosynthesis*

INTRODUCTION

Homoserine was first chemically synthesized by Fischer and Blumenthal in 1907.[1] L-Homoserine ($C_4H_9NO_3$), also known as 2-amino-4-hydroxybutyric acid, is a tetracarbon amino acid belonging to the aspartate family, and is a non-proteinogenic amino acid,[2] serving as a crucial precursor for the biosynthesis of L-threonine, L-methionine and other essential amino acids, which are widely used in the fields of medicine, chemical industry and agriculture[3−6] (Figure 1). It can be used as a precursor to synthesize important compounds such as 1,3-propanediol,[7] 2,4-dihydroxybutyric acid,[8] L-homoserine lactone,[9] isobutanol,[9] L-cysteine,[10] etc. Using L-homoserine as a chiral source, the novel herbicide L-glufosinate was also obtained through chemical processes, resulting in L-glufosinate with a total yield of 76.5% and an enantiomeric selectivity of 93.8%. This synthetic route exhibits a relatively uncomplicated and feasible operation, with promising prospects for industrialization. L-Homoserine is also an essential and functional amino acid, capable of serving as an antifungal medication and possessing the potential as a skin hydrating agent.[11] It can be used as a feed additive in agriculture to improve the resistance of plants to diseases,[12] exhibiting similar functions as L-threonine.[13] Thus, L-homoserine holds significant significance in the realms of biochemistry, pharmaceuticals, cosmetics, and related sectors. Consequently, the market's incessant growth necessitates an urgent expansion of the industrial manufacturing of L-homoserine.[14]

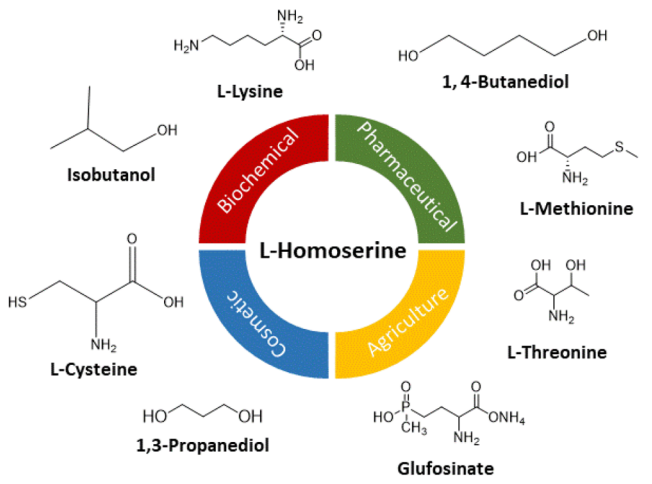


Figure 1. Representative examples of L-homoserine applications.

Traditional production of L-homoserine mainly relies on chemical synthesis. There are two chemical approaches: dissolving L-methionine in water and placing it together with CH_3I in a flask, followed by steps such as vacuuming,

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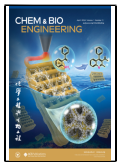

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Table 1. Metabolic Engineered *E. coli* Strains for L-Homoserine Production

Chassis	Genotype	Titer (g/L)	Volumetric productivity (g/L/h)	Yield (g/g)	Ref
E. coli W3110	ΔlysA,ΔmetA,ΔthrBC,ΔiclR,ΔgltA,ΔpykA,ΔpykF/Pkk-miniPtac-thrA	35.8 (7 L fermenter)	0.82	0.35	2
E. coli W3110	ΔlacI,ΔlysA,ΔmetA,ΔthrBC,Δtdcc/pBRmetl-pNrhtA	39.54 (15 L fermenter)	0.9	0.29	32
E. coli W3110	ΔlacI,ycgH::P_{trc}-thrA^{fbr},P_{thrABC}::P_{fliC}ydeU::P_{trc}-thrA^{fbr},yjhE::P_{trc}-thrA^{fbr},P_{ppc}::P_{trc},ylbE::P_{trc}-aspC,ycdN::P_{trc}-aspA,tfaD::P_{trc}-thrA^{fbr},yeeL::P_{trc}-ppc,ycjV::P_{trc}-lysCfbr cgl ,yjiP::P_{lpp}-rhtA,ilvG::P_{trc}-pntAB,ygaY::P_{trc}-pntAB,yeeP::P_{trc}-asd_{tmo},yghX::P_{trc}-adh_{pae}	85.29 (5 L fermenter)	1.78	0.43	33
E. coli W3110	ΔmetJ,ΔmetI,ΔmetB,Trc-metL,ΔthrB,ΔmetA,Trc-thrA,ΔlysA,ΔlacI::Trc-rhtA,Trc-rhtA,Trc-eamA,ΔiclR,ΔptsG, ΔgalR,Trc-glk,Trc-gltB/pACYC-pyc^{P458S}-thrA^{G433R}-lysC	37.57 (5 L fermenter)	0.35	0.31	56
E. coli BW25113/F[45]	ΔmetAΔlysAΔthrBΔlacIΔsthA::P_{tac}-pntABΔldhAΔpoxBΔpflBΔfliK::P_{tac}-rhtBΔyeeJ::P_{119}-rhtBΔptsG::P_{119}-glkΔgalR::P_{119}-zglfΔompT::P_{tac}-ppcΔiclRΔyjiV::P_{tac}-aspC-gdhApS95s-thrA*-asd-aspA-RBS2800	84.1 (5 L fermenter)	1.96	0.5	44
E. coli W3110	P_{thrB}::P_{fliC}ylbe::P_{trc}-thrA^{fbr},yjit::P_{trc}-ppc,mbhA::P_{trc}-aspA,rph::P_{trc}-thrA^{fbr}yjip::P_{trc}-thrA^{fbr},gapC::P_{trc}-pntAB, ygay::P_{lpp}-rhtA	60.1 (5 L fermenter)	1.25	0.42	34
E. coli W3110	ΔlysAΔthrBΔmetAΔlacIΔldhAΔadhE ΔpflBΔptsGΔP_{galP}::P_{lac}ΔP_{ppc}::P_{trc}ΔiclRΔarcAΔP_{aspA}::P_{trc}ΔP_{glk}::P_{lac}ΔP_{asd}::P_{trc}ΔgalP::glf/pBbA1K harboring metL gene, controlled by grac/RBS3 promoter/pTrc99A harboring rhtA gene, controlled byPrhtA promoter	110.8 (2 L fermenter)	1.82	0.62	50
E. coli W3110	ΔlysAΔmetAΔthrBC-ppc(trc)-thrA(trc)-asd(trc)-pntAB(trc)-rhtA(trc) containing pKK-metL-hok2	44.4 (5 L fermenter)	0.93	0.21	49

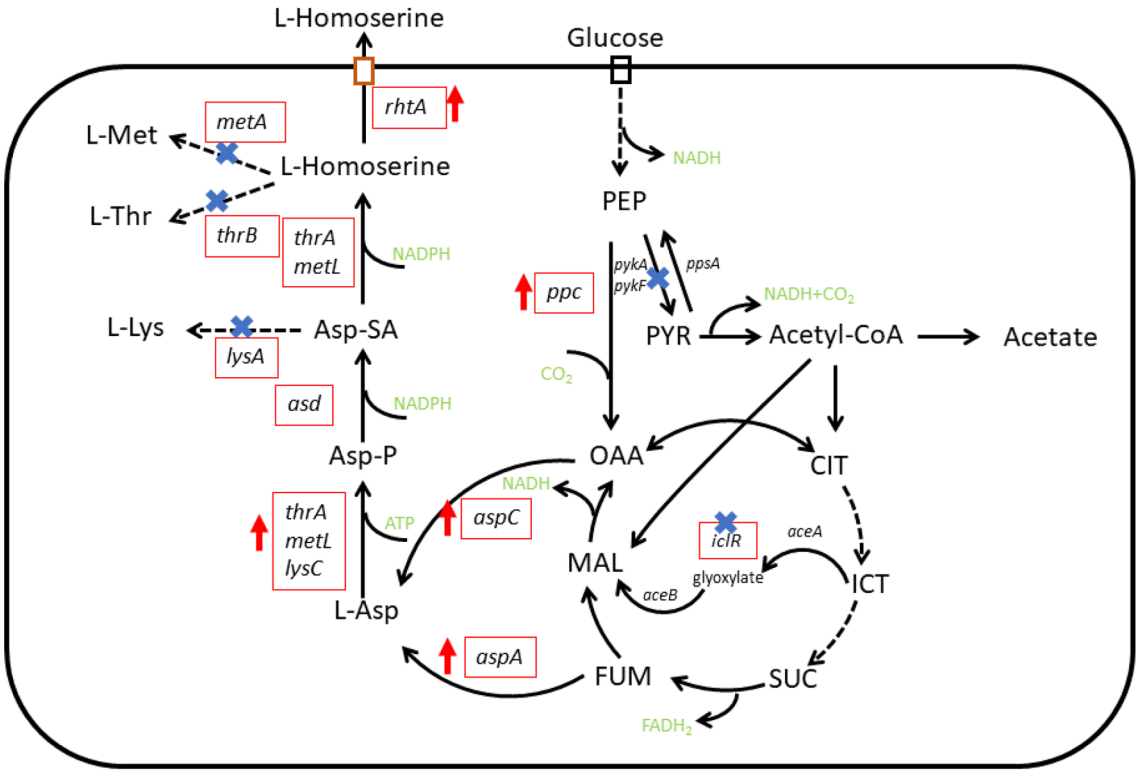

Figure 2. Metabolic pathway of L-Homoserine in *E. coli*.. Abbreviations: PEP, phosphoenolpyruvate; PYR, pyruvate; OAA, oxaloacetate; CIT, citrate; ICT, isocitrate; SUC, succinate; FUM, fumarate; MAL, malate; L-Asp, L-aspartate; Asp-P, aspartyl phosphate; Asp-SA, aspartyl semialdehyde; L-Lys, L-lysine; L-Thr, L-threonine; L-Met, L-methionine; *ppc*, encoding phosphoenolpyruvate carboxylase; *thrA*, encoding aspartate kinase I and homoserine dehydrogenase I; *met*L, encoding aspartate kinase II and homoserine dehydrogenase II; *lysC*, encoding aspartate kinase III; *iclR*, encoding IclR family transcriptional regulator; *aceA*, encoding isocitrate lyase; *aceB*, encoding malate synthase; *ppsA*, encoding phosphoenolpyruvate synthase; *pykA*/*pykF*, encoding pyruvate kinase; *aspA*, encoding aspartate oxidase; *aspC*, encoding aspartate transaminase; *asd*, encoding aspartate-semialdehyde dehydrogenase; *thrB*, encoding homoserine kinase; *metA*, encoding homoserine-O-succinyltransferase; *lysA*, encoding diaminopimelate decarboxylase; *rhtA*, encoding inner membrane transporter. The solid line represents a one-step reaction and the dotted line represents a multi-step reaction.

condensing, refluxing, purifying, and drying, resulting in a yield of 65.8% for the product homoserine of 65.8%. Alternatively, by combining γ-butyrolactone with PBr_3 in a flask, heating, reacting, extracting, and recrystallizing, one can obtain homoserine in a yield of 70%. However, the homoserine synthesized through chemical approaches is a racemic mixture. To obtain a pure form of L-homoserine, complex separation and purification procedures are necessary, significantly increasing the production cost of L-homoserine.[15]

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Nevertheless, the production of L-homoserine by microbial fermentation has the advantages of mild reaction conditions and is environment-friendly, which is the future technology for industrial production of L-homoserine.[16,17] Since Plachý et al. first reported that L-homoserine could be produced by microbial fermentation from *Corynebacterium* SP. in 1985,[18] people have aroused great interest in microbial production of L-homoserine and its derivatives.[19−22] Until now, L-homoserine has been successfully produced in *Escherichia coli* (*E. coli*) (Table 1) and *corynebacterium glutamicum* (*C. glutamicum*).[23,24] Although *C. glutamicum* has made some progress in fermenting and producing L-homoserine, its application is limited due to its long fermentation cycle and relatively low current yield. On the other hand, *E. coli* has been chosen as the host strain by most researchers due to its clear genetic background, rapid reproduction, efficient gene manipulation, and relatively simple cultivation conditions.[25−27] Furthermore, *E. coli* currently has a higher yield in the fermentation production of L-homoserine compared to *C. glutamicum*. However, in the process of industrialization of L-homoserine production by microbial fermentation, it is necessary to further increase the yield and reduce the economic cost. With the continuous development and promotion of system metabolic engineering technology, the production of L-homoserine has been a breakthrough progress.

This Review summarizes recent advances in the systematic metabolism strategies used for L-homoserine production in engineered *E. coli* strains, including genetic modifications of biosynthetic pathways and transport systems and also the existing bottlenecks and perspectives.

THE METABOLIC PATHWAY OF L-HOMOSERINE IN *E. COLI*

The Gram-negative, nonsporulating bacterium *E. coli* is the most commonly used microbial cell factory for industrial fermentation of L-homoserine. A variety of systems' metabolic engineering tools and strategies have been developed for *E. coli* and used to construct engineered *E. coli* strains suitable for industrial-scale production of valuable chemicals and materials.[16,27−29] L-Homoserine belongs to the L-aspartate family and is synthesized from L-aspartate. *E. coli* owns a natural L-homoserine metabolic pathway, but there are also competing pathways, including the L-lysine and L-threonine synthetic pathways. L-Homoserine may be internalized to biosynthesize the proteinogenic amino acids L-methionine and L-threonine intracellularly, or alternatively, it can be transported extracellularly.[30]

Glucose is always used as the carbon source in the *E. coli* metabolic pathway for L-homoserine synthesis (Figure 2). It is first metabolized by the phosphotransferase system (PTS) and then converted into phosphoenolpyruvate (PEP) through the Embden-Meyerhof pathway (EMP). A portion of PEP is converted to pyruvate (PYR). PYR is further metabolized to acetyl-CoA, which entered the tricarboxylic acid (TCA) cycle, and a part of acetyl-CoA is converted to oxaloacetate (OAA), the precursor of L-aspartate. OAA is converted to L-aspartate through an aspartate transaminase (encoded by *aspC*) and then transforms to aspartyl phosphate through an aspartate kinase (AK, encoded by *thrA*, *metL*, and *lysC*). Aspartyl phosphate is dehydrogenated by an aspartate-semialdehyde dehydrogenase (encoded by *asd*) to form aspartyl semialdehyde, which is finally converted to L-homoserine by the homoserine dehydrogenase.

METABOLIC ENGINEERING STRATEGIES USED FOR L-HOMOSERINE PRODUCTION IN *E. COLI*

1. Blocking the Competing and Degrading Pathways of L-Homoserine. The accumulation of L-homoserine is difficult in wild-type *E. coli*, because it is a metabolic intermediate that will be further metabolized to L-threonine and L-methionine.[2] To impede the deterioration of L-homoserine and foster its accumulation, it is imperative to obstruct these two pathways of degradation. The biosynthetic pathways of both L-lysine and L-homoserine compete for aspartyl semialdehyde (Figure 2), and to smoothly redirect carbon flux from aspartate toward L-homoserine, the *lysA* encoding diaminopimelate decarboxylase needs to be knocked out, thereby blocking the L-lysine biosynthetic pathway.[31] Studies found that three knocked out genes (*metA*, *thrB*, and *lysA*) from *E. coli* W3110 obtained a L-methionine, L-threonine, and L-lysine auxotroph strain, which achieved 0.12 g/L accumulation of L-homoserine in a shaking flask culture. Then the transport system was further modified and *metL* gene was overexpressed, 39.54 g/L of L-homoserine was obtained in a 15 L fermenter, and the yield reached 0.29 g/g glucose.[32] However, it was not competitive in the industry because exogenous supplementation of essential amino acids was required for cell growth and metabolism of target products. Based on this, Cai et al. tried to finetune the metabolic flux distribution of L-homoserine production strains by changing the promoter strengths and found that the effect of directly knocking out *thrB* to block the degradation pathway was similar to that of using self-regulated promoter P_{fliC} to downregulate *thrB* expression.[33] Zhang et al. constructed a non-induced, non-auxotrophic, plasmid-free *E. coli* chassis, and the titer of L-homoserine reached 60.1 g/L, laying a certain research foundation for industrial production of L-homoserine.[34]

2. Overexpression of Key Enzymes and Elimination of Feedback Inhibition. Three enzymes, aspartate kinase (AK), aspartate-semialdehyde dehydrogenase, and homoserine dehydrogenase, are involved in the biosynthesis of L-homoserine from L-aspartate.[35] The regulatory mechanisms of these three key enzymes are different,[36] and another key point is that many genes involved in L-homoserine synthesis are inhibited by the feedback of metabolites.

AK is the most vital pivotal enzyme in the L-homoserine synthesis pathway. It phosphorylates L-aspartate to aspartyl phosphate, which allows carbon flux into the L-aspartate pathway. In *E. coli*, there are three isozymes of AK, named AK I, AK II, and AK III, encoded by *thrA*, *metL*, and *lysC*, respectively.[37] Liu et al. increased the titer of L-homoserine by 2.5-fold through overexpression of *thrA*, indicating that the overexpression of *thrA* increased the carbon flux to the biosynthetic pathway of L-homoserine, thus enhancing the production of L-homoserine.[2] Cai et al. also found that *thrA* transcription level was significantly up-regulated when P_{trc}-$thrA^{fbr}$ was increased to three copies, so as to enhance the synthesis flux of L-homoserine.[33] AK I and AK II are bifunctional enzymes with both AK and homoserine dehydrogenase activities. The catalytic activities of AK I and AK III are feedback inhibited by L-threonine and L-lysine, respectively.[5] And the expression of AK II is tightly regulated by the MetJ repressor and adenosylmethionine corepressor in response to L-methionine levels.[5,17] A number of mutants obtained by protein engineering that eliminate feedback inhibition have been reported.[38] Lee et al. eliminated the feedback inhibition of AK

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I and AK III by replacing the 1034th C with T in *thrA* ($ThrA^{S345F}$) and the 1055th C with T in *lysC* (Lys^{T342I}).[29]

Aspartate-semialdehyde dehydrogenase is encoded by *asd*, which catalyzes the dehydrogenation of aspartyl phosphate to form aspartyl semialdehyde. There is currently no evidence of feedback inhibition by the metabolites of this enzyme.

Homoserine dehydrogenase, another key enzyme in this pathway, regulates the carbon flux to L-homoserine synthesis and competes with diaminopimelate decarboxylase (the catalytic branch of L-lysine synthesis) for the substrate aspartyl semialdehyde. As mentioned above, *thrA* and *MetL* encode homoserine dehydrogenase I and homoserine dehydrogenase II. A low expression level of homoserine dehydrogenase II is found in *E. coli*, and studies about their expression levels are rarely performed.[37]

3. Strengthening the Precursor Pools for L-Homoserine Biosynthesis. L-Aspartate is a key precursor for L-homoserine biosynthesis, and many efforts have been done on upregulating the precursor L-aspartate.[39] In *E. coli*, L-aspartate can be obtained from OAA catalyzed by aspartate transaminase (AspC, encoded by *aspC*) or synthesized from fumarate by aspartate ammonia lyase (AspA, encoded by *aspA*).[40−42]

The reaction facilitated by AspC is invariably accompanied by the reduction of α-ketoglutarate to L-glutamate.[43] L-Aspartate can be synthesized by fixing 2 mol of CO_2 through the AspC pathway, and the highest theoretical yield of L-homoserine is 2 mol/mol glucose (eq 1).[44] From this equation, it is found that to achieve the highest yield of L-homoserine, 6 mol of NADPH was needed. However, 1 mol of glucose consumption only produces 2 mol of NADH, and the NAD(P)H was severely insufficient, so the yield of L-homoserine could not reach the highest theoretical yield, leading to low atomic economy.

$$\text{Glucose} + 6\text{NADPH} + 2CO_2 = 2\text{L-homoserine} + 2\text{NADH} \quad (1)$$

AspA catalyzes the synthesis of L-aspartate from fumarate produced in the TCA cycle, which is known as the AspA pathway and can effectively replace the AspC pathway.[44] From this way, the highest theoretical yield of L-homoserine is 1 mol/mol glucose (eq 2).[44] The equation revealed that there is a large amount of NAD(P)H remaining during the AspA pathway.

$$\text{Glucose} + 2\text{NADPH} = \text{L-homoserine} + 2CO_2 + 5\text{NADH} + FADH_2 \quad (2)$$

Once these two pathways were combined, the equilibrium of the reductive ability is well-maintained. The CO_2 released from the AspA pathway can be fixed by the AspC pathway. The theoretical yield of L-homoserine is 1.5 mol/mol glucose (eq 3),[44] without any loss of carbon atoms. A balanced redox metabolic network is needed for L-homoserine production, and a synergistic strategy can be employed to obtain a relatively balanced NAD(P)H, thereby achieving high productivity of L-homoserine.

$$2\text{Glucose} + 8\text{NADPH} = 3\text{L-homoserine} + 7\text{NADH} + FADH_2 \quad (3)$$

When *aspC* and *aspA* were overexpressed separately, the titers of L-homoserine reached 22.6 and 54.4 g/L, respectively. To achieve a balance between oxidation and reduction, these two pathways were optimized by incorporating optimization strategies from other metabolic pathways, and the titer of L-homoserine was elevated to a remarkable 81.2 g/L. Ribosomal Binding Sites (RBSs) with different strengths were further designed to regulate the expression strengths of *aspA*. Finally, the highest titer of L-homoserine was 84.1 g/L and the yield was 0.5 g/g glucose using the medium-strength RBS2800.[44] Piao et al. also constructed an *E. coli* chassis synthesizing the precursors L-aspartate and its derivate β-alanine with high stoichiometric yield by overexpressing *ppc* and *aspC* and establishing a cofactor self-sufficient system to improve the efficiency of AspC-catalyzing reaction.[45]

4. Enhance the Carbon Flow from PEP to L-Homoserine. The TCA cycle and EMP pathway are the main central metabolic pathways in *E. coli*, dissimilating carbon sources into PEP, PYR, and acetyl-coA to provide energy for cell growth.[46,47] The TCA cycle plays an important role in cell metabolism by providing precursors ATP and $FADH_2$ for biosynthesis. In the EMP pathway, the products PEP and PYR generate acetyl-CoA and OAA to enter the TCA cycle.[48] OAA, PEP, and PYR control carbon fluxes in the L-homoserine synthetic pathway. Anabolic engineering of OAA, PEP, and PYR is important for the biosynthesis of L-aspartate family amino acids (AFAAs) during central metabolism.[30,49]

Increasing carbon flow from PEP to OAA may be one of the key strategies to break the bottleneck in L-homoserine synthesis, as OAA is a crucial precursor for the biosynthesis of AFAAs.[35] In *E. coli*, OAA is mainly obtained by phosphoenolpyruvate carboxylase (encoded by *ppc*) catalyzed from PEP. The most commonly-used strategy to increase OAA is to overexpress *ppc* in engineered strains to enhance the carbon flux from PEP to OAA.[2,32,33] However, the PTS system couples glucose transport/phosphorylation with the conversion of PEP to PYR in *E. coli*, resulting the efficient glycolysis but inefficient transformation from PEP to OAA. Therefore, Vo et al. deleted the *ptsG*, so the PTS system deficient strain, which blocks the glycolysis process and improved the carbon flux from PEP to OAA. But due to the decreased glucose transportation, both strain growth and L-homoserine production were reduced. This issue was subsequently resolved by upregulating the expression of galactose permease (encoded by *galP*) through replacing the original promoter of *ppc* with the strong promoter P_{trc}, which increased the L-homoserine yield from 0.32 to 0.37 mol/mol.[50]

PYR and PEP connect the metabolic pathways of amino acids, carbohydrates, and lipids, so the regulation of PYR is critical for cell metabolism.[51,52] Pyruvate kinase converts PEP to PYR, and there are two PYR isoenzymes in *E. coli*, namely, PYR I and PYR II (encoded by the *pykF* and *pykA*).[51,53] In order to determine whether the deletion of *pyk* would affect the production of L-homoserine, many researchers found that the production of L-homoserine had a certain increase after deleting *pykF* and *pykA*.[2] It has also been reported that PYR II contributes little to the carbon flux distribution through PYR *in vivo*.[54] When the *pykF* was knocked out, the flux of EMP decreased from 65% to 20%, and the PPP equivalent increased from 34% to 79%.[55]

As a complementary pathway in the TCA cycle, the glyoxylate cycle pathway directly converts isocitrate to fumarate and malate, accelerating the conversion of central carbon metabolism to target products.[44] There is a transcription factor IclR (encoded by *iclR*) in the glyoxylate cycle pathway, which inhibits the expressions of *aceA* and *aceB* encoding malate synthetase and isocitrate lyase, respectively.[30] Therefore, the knockout of *iclR* removes the negative feedback on the transcription of key genes in the glyoxylate cycle pathway. Results showed that diminishing the futile cycle within the TCA cycle through knockout of *iclR*

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promoted glyoxylate shunt without any other negative impact, thereby increasing OAA supply and L-homoserine production.[2,56]

5. Modification of the Transport System for Enhancing L-Homoserine Efflux. The intracellular accumulation of the target product triggers product inhibition. To prevent this phenomenon, efficient extracellular transportation of the amino acid product is imperative. Modification of the transport system can effectively increase the efflux of the product and improve the survival rate of the cell.[57] Studies have shown that L-homoserine inhibits the growth of *E. coli*, so the intracellular concentration of L-homoserine needs to be kept at a low level (<13 mM).[23,58]

In 1974, Templeton et al. proved for the first time that L-threonine and L-homoserine share a transport system. However, due to the lack of in-depth research, no specific transporters of these two amino acids were identified at that time.[59] Until 1990, Sumantran et al. found that L-homoserine could be transported through tdcC transporter in *E. coli* by comparing L-homoserine utilization of overexpression or inactivation of *tdcC*.[60] Subsequently, Kruse et al. identified RhtB (encoded by *rhtB*) and RhtC (encoded by *rhtC*) as two transporters of L-homoserine homologous and L-threonine.[61] Livshits et al. discovered a new L-homoserine transporter, RhtA (encoded by rhtA), which belongs to a large family of transporters. Studies found that upregulating the expression of rhtA significantly improved the transport of L-homoserine.[62] The bacterial growth and production were greatly improved by overexpression of rhtA with a stronger promoter $P_{lpp.}$[33] When the two copies of rhtA were overexpressed with another transporter eamA, L-homoserine production was higher than that when only one of the two transporters was expressed.[56]

Transporters are one of the research hotspots of L-homoserine biosynthesis. So far, some transporters of L-homoserine have been found, including RhtA, RhtB, EamA, BrnFE, etc. The identification of novel transporters assumes a pivotal role and holds great significance in attaining heightened yields of L-homoserine. It also provides a favorable solution to solve the problem of how to improve host survival and increase yield under a high concentration of L-homoserine.

6. Recycle the Coenzyme NADPH. In the metabolic process of bacteria, coenzymes such as NADPH/$NADP^+$ are important mediators of intracellular energy transfer and provide redox carriers for cell biosynthesis and decomposition reactions.[63,64] Therefore, maintaining a cellular redox balance is an important prerequisite for maintaining normal cell metabolism and achieving efficient production of target products.[65,66]

In the biosynthesis of L-homoserine, the reaction from aspartyl phosphate to L-homoserine requires the participation of NADPH, so maintaining the balance of NADPH is likely to achieve high production of L-homoserine.[44] Previous studies have shown that overexpression of the *pntAB* encoding pyridine nucleotide transferase effectively promoted the regeneration of NADPH, which has been successfully applied in the metabolic engineering of amino acids such as L-lysine to improve the yield of target products.[67,68] In the modification of L-homoserine-producing strains, overexpression of *pntAB* is also one of the common strategies to increase NADPH.[33,49] Some researchers have attempted to introduce heterologous NADH-dependent glutamate dehydrogenase, mediating the regeneration of L-glutamate from α-KG and coupling this reaction with *aspC*. It was found that the total amount of L-homoserine increased by 23.9% after introducing this NADH-dependent dehydrogenase, indicating that the synergistic utilization of NADPH and NADH is effective for the overproduction of L-homoserine.[33]

CONCLUSION AND OUTLOOK

L-Homoserine is an important functional amino acid and has a high industrial application value. In this Review, the metabolic strategies employed in *E. coli* for the elevated synthesis of L-homoserine are comprehensively outlined, thereby establishing a solid basis for the industrialization thereof.

The current strategies for modifying *E. coli* to produce L-homoserine are not yet perfect. In-depth research could be carried out on different aspects. A comprehensive analysis and optimization of the cellular metabolic synthesis pathways can be achieved via advanced synthetic biology techniques, metabolic network analysis, and transcriptomics analysis. The rationally designed metabolic pathways can be conducted to effectively coordinate the production of L-homoserine and its intermediates, as well as establish a harmonious relationship between L-homoserine production and microbial growth. Furthermore, for the accumulation of intermediates by blocking degradation and competing pathways, the strain results in deficiencies and impacts cell growth. It necessitates the addition of nutrients in large-scale fermentation, thereby increasing production costs. The current research mainly focuses on the genetic modification of L-homoserine production strains, with minimal research on the influence of fermentation conditions. Consequently, the next step should involve systematic optimization of the fermentation process. Moreover, *C. glutamicum* has successfully achieved the efficient production of L-lysine, L-glutamic acid, L-threonine, etc. It should also have great potential for the efficient production of L-homoserine. However, there is limited research on the production of L-homoserine by *C. glutamicum*. Therefore, one of the future directions is to employ metabolic engineering strategies and improve the production capacity of L-homoserine in *C. glutamicum*. Additionally, it is also crucial to develop other non-model chassis for the efficient production of L-homoserine. For instance, using the fastest-growing bacterium *Vibrio natriegens* may greatly shorten the fermentation time.[69] We believe that with the continuous development of synthetic biology and metabolic engineering technologies, the biosynthesis and industrialization of L-homoserine is expected.

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Y.J.S. wrote the paper. J.Q.X., L.R.Y. and J.P.W. read and corrected the manuscript. All authors have read and approved the final manuscript. All authors read and approved the final manuscript.

Notes

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■ ABBREVIATIONS

E. coli, *Escherichia coli*; *C. glutamicum*, *Corynebacterium glutamicum*; PTS, the phosphotransferase system; PEP, phosphoenolpyruvate; EMP, the Embden−Meyerhof pathway; PYR, pyruvate; TCA, the tricarboxylic acid; OAA, oxaloacetate; AK, aspartate kinase; RBSs, ribosomal binding sites; AFAAs, L-aspartate family amino acids

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